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Field evaluation of effects of transgenic *cry1Ab/cry1Ac*, *cry1C* and *cry2A* rice on *Cnaphalocrocis medinalis* and its arthropod predators

XU XueLiang[1,2], HAN Yu[1], WU Gang[1], CAI WanLun[1], YUAN BenQi[1], WANG Hui[1], LIU FangZhou[1], WANG ManQun[1] & HUA HongXia[1*]

[1]*Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China;*
[2]*Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China*

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The impacts of transgenic *Bt* rice on target pests and their predators need to be clarified prior to the commercialization of *Bt* rice. In this study, the percentages of folded leaves of three transgenic *Bt* rice lines and non-transgenic parental rice line caused by *Cnaphalocrocis medinalis* were studied over two successive growing seasons. In addition, the population densities, relative abundance and population dynamics of *C. medinalis* and four species of its natural arthropod predators were investigated at three sites in China. The results showed that rice line significantly affected the percentages of folded leaves and population densities of *C. medinalis* larvae. Significantly higher percentages of folded leaves were observed on the non-transgenic rice compared with the three transgenic *Bt* rice on most sampling dates. Significantly higher densities of *C. medinalis* larvae and higher relative abundance of *C. medinalis* within phytophages were found on non-transgenic rice compared with three transgenic *Bt* rice at different sites across the study period. The population dynamics of *C. medinalis* larvae were significantly affected by rice line, rice line×sampling date, rice line×year, rice line×sampling date×year. However, there was little, if any, significant difference in the relative abundance, population density and population dynamics of the four arthropod predators between the three *Bt* rice lines and non-transgenic rice. The results of this study indicate that the *Bt* toxin in transgenic *Bt* rice can effectively suppress the occurrence of *C. medinalis*, but has no significant effects on the occurrence of the four predatory arthropod species.

transgenic *Bt* rice, *Cnaphalocrocis medinalis*, population dynamics, percentages of folded leaves, natural arthropod predator

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Rice is the staple food crop in Asia. Significant losses of this valuable crop occur every year as a result of the infestations of stem borers and leaffolders, two groups of lepidopteran rice pests [1,2]. Traditionally these pests were controlled by poisonous chemicals. However, the use of large amounts, as well as the long-term applications, of these chemicals resulted in environmental contamination and significantly increased the costs of rice production. In addition, lepidopteran pests quickly developed resistance to the pesticides, which reduced the efficacy of chemical control [3,4]. Researchers were therefore prompted to seek more economic and environmentally friendly strategies for controlling lepidopteran pests. *Bacillus thuringiensis* (*Bt*) insecticidal δ-endotoxin has been used as a biological insecticide for more than 50 years, and it is now possible to introduce different *Bt* genes into rice. The transgenic rice plants ex-

*Corresponding author (email: huahongxia@mail.hzau.edu.cn)

pressing *cry1Ab* or *cry1Ab/cry1Ac* develop resistance to stem borers and leaffolders, resulting in effective control of target lepidopteran pest infestations [5–8]. The deployment of transgenic *Bt* rice resulted in yield increases of 6%–9%, and an 80% reduction in insecticide use, compared with conventional rice varieties [9].

The leaffolder, *Cnaphalocrocis medinalis*, is a widely distributed lepidopteran pest on rice, and the main target herbivore pest of transgenic *Bt* rice. Prior to the commercial release of *Bt* rice, the resistance of transgenic *Bt* rice on target lepidopteran pests should be evaluated. Thus, most published studies have focused on the effects of transgenic *Bt* rice on the development and population dynamics of such target lepidopteran pests [10–13]; however, little is known about the potential prey-mediated effects of transgenic *Bt* rice on non-target predators through tritrophic interactions. There are only a few reports of the effects of transgenic *Bt* rice on predators of target lepidopteran pests; for example, Chen *et al.* reported that Cry1Ab protein derived from transgenic *cry1Ab* rice plants could be detected in the spider *Pirata subpiraticus*, a predator of *C. medinalis*, although the concentration of Cry1Ab detected did not increase as its preying time increased. *P. subpiraticus* lacks binding receptors for Cry1Ab in its midgut, which could explain why there was no significant difference in the survivorship and fecundity of *P. subpiraticus* preying on *Bt* rice-fed *C. medinalis* compared with those preying on non-*Bt* rice-fed *C. medinalis*. However, the developmental time of *P. subpiraticus* was significantly longer when it preyed on *Bt* rice-fed *C. medinalis* compared with those feeding on non-*Bt* rice-fed prey. In addition, a three-year field trial indicated that *Bt* rice did not significantly affect the density of *P. subpiraticus* [14]. When *C. medinalis* larvae fed with transgenic *cry1Ab* rice were used as prey, the presence of the Cry1Ab had no marked effect on the predation rate of *P. subpiraticus* [15]. The population dynamics of five dominant spider species (*Tetragnatha maxillosa*, *Dyschiriognatha quadraimaculata*, *Ummeliata insecticeps*, *Pardosa pseudoannulata* and *P. subpiraticus*) in transgenic *cry1Ab/cry1Ac* rice plots were similar to those in non-transgenic control plots, as were the population densities of these species [16]. All these reports are about the impacts of Cry1Ab and Cry1Ab/Cry1Ac on non-target predators of *C. medinalis*, but up to the present, there is no literature about the impacts of Cry2A and Cry1C *Bt* toxin on population density and dynamics of predators of *C. medinalis* in rice paddy fields.

Hylyphantes graminicola, *P. pseudoannulata*, *U. insecticeps* and *P. fuscipes* are the main arthropod predators of *C. medinalis* in rice fields [16–19]. In the current study, three transgenic *Bt* rice lines (containing *cry1C*, *cry2A* and *cry1Ab/cry1Ac*) and a non-transgenic control were planted at three sites in Hubei Province, China over a two-year growing season. Arthropods were sampled by vacuum-suction machine and plant dissection, and identified to species level. The suppressive effects of the transgenic rice on infestations of *C. medinalis*, the population density, relative abundance and population dynamics of the four predatory species of *C. medinalis* were assessed via field trials.

1 Materials and methods

1.1 Plant material

Three transgenic *Bt* rice lines (TT51, T1C-19 and T2A-1) and the non-transgenic parental *indica* rice line Minghui 63 were selected for field evaluation. TT51 is a transgenic rice line expressing a *Bt* fusion gene derived from *cry1Ab* and *cry1Ac* under the control of the rice *actinI* promoter [8]. T2A-1 expresses a synthesized *cry2A* gene, whereas T1C-19 expresses a synthesized *cry1C* gene, both driven by the maize *ubiquitin* promoter [12,20]. All the rice lines were gifted by Lin YongJun, National Key Laboratory of Crop Genetic Improvement, Wuhan, China.

1.2 Field experiment design

Field experiments were conducted during the 2009–2010 growing season at three different sites, where field trials of *Bt* rice were permitted, in Hubei Province, China. The first site was in a suburb of Xiaogan (113.54°E, 30.56°N), the second was in a suburb of Suizhou (113.22°E, 31.42°N) and the third was in a suburb of Wuxue (115.33°E, 29.51°N). The layout of the plots in the field followed a randomized block design with three replications for each treatment. Each experimental plot was 20 m×25 m, surrounded by 1 m wide unplanted border. The entire experimental field was bordered by five rows of non-transgenic control plants. In each of the study years, *Bt* rice and non-transgenic control rice were sown in early May and transplanted one month later. Seedlings were manually transplanted with one seedling per plant, with 13.3 cm between plants within a row and 29.9 cm between rows. Agronomic practices for growing rice, such as fertilization and irrigation, were followed by local farmers, except that no insecticides were applied at any sites.

1.3 Sampling by plant dissection and vacuum-suction machine

Thirty rice hills were randomly selected to investigate the number of larvae and pupae of *C. medinalis*, and damage caused by *C. medinalis*. The total number of leaves from the 30 selected rice hills and the total number of leaves with rolls or visible scrapes were recorded to evaluate the damage caused by *C. medinalis*. The leaves infested by *C. medinalis* were dissected to count the number of larvae or pupae presented.

Sampling of adult *C. medinalis*, as well as predators of *C. medinalis*, was done as described by Chen *et al.* [21]. Arthropods at all three field sites were collected using a vac-

uum-suction machine, based on a description by Carino *et al.* [22] and supplemented by a square sampling box (50 cm length×50 cm width×90 cm height) with a metal frame and enclosed by Mylar film. The sampling box could enclose six rice hills at a time. Samples were collected every 10–15 d, starting one month after transplantation and continuing until the rice was ripe (i.e., as measured by grain maturity and harvest). On each sampling date, a square sampling box was placed at random along the diagonal line of each test plot at each site, with five sub-samples per plot. The sample location in each plot was marked with a bamboo stake to avoid resampling at the same location. Arthropods inside the frame enclosure were collected using the vacuum-suction machine at each sampling location for 5 min, and were preserved in 75% ethanol. All samples were taken back to the laboratory and identified to species level.

1.4 Data analysis

Population density, relative abundance and population dynamics collected by vacuum-suction and dissection were used to evaluate the impacts of the three transgenic *Bt* rice on *C. medinalis* and its four predators. The arthropods were divided into five guilds: phytophages, parasitoids, predators, detritivores, and others. The relative abundance of each arthropod species was calculated as number of the species/total number of the guild to which it belongs. The population density of each species was the seasonal means captured by vacuum-suction. Population dynamics of each species were measured by means at each sampling date. Statistical analyses were performed with STATISTICA 7 [23]. All count data with percentages were transformed to arcsine-square root values transformed before statistical analysis.

2 Results

2.1 Percentages of folded leaves

Rice line, sampling date, year, rice line×sampling date, rice line×year, sampling date×year, rice line×sampling date× year significantly affected the percentages of folded leaves in the four rice fields (P<0.05) at the three experiment sites (Table 1). At Xiaogan, significantly higher percentages of folded leaves caused by *C. medinalis* were observed on non-transgenic Minghui 63 compared with the three transgenic *Bt* rice lines at all sampling dates in 2009 and 2010 (P<0.01) except for those recorded at 98 days after transplanting (DAT) in 2009 (P=0.0796; Table 2). At Suizhou, in 2009 and 2010, the percentages of folded leaves caused by

Table 1 Three-way ANOVAs at three sites of the effects of rice line, sampling date, investigation year, and their interactions on the measured indexes of rice, herbivores and arthropod predator species (P-value)

Site	Measured indexes	Rice line[a)]	Sampling date[b)]	Year[c)]	Rice line×sampling date[d)]	Rice line×year[e)]	Sampling date×year[f)]	Rice line×sampling date×year[g)]
Xiaogan	Percentages of folded leaves	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Population dynamics of *C. medinalis* larvae	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Population dynamics of *C. medinalis* adults	0.0034	0.0072	0.0950	0.0786	0.0002	<0.0001	0.4171
	Population dynamics of *H. graminicola*	0.5478	0.0001	0.0002	0.6166	0.6698	<0.0001	0.5917
	Population dynamics of *U. insecticeps* per	0.3826	<0.0001	0.0006	0.5573	0.8090	0.6076	0.9901
	Population dynamics of *P. pseudoannulata*	0.6083	<0.0001	<0.0001	0.8772	0.5910	0.0051	0.8980
	Population dynamics of *P. fuscipes*	0.0333	0.8968	0.0001	0.3804	0.4484	0.3959	0.5971
Suizhou	Percentages of folded leaves	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Population dynamics of *C. medinalis* larvae	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Population dynamics of *C. medinalis* adults	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
	Population dynamics of *H. graminicola*	0.0627	<0.0001	0.2691	0.9802	0.2549	0.0007	0.4479
	Population dynamics of *U. insecticeps* per	0.5817	0.0052	0.2674	0.2039	0.3179	0.0098	0.6854
	Population dynamics of *P. pseudoannulata*	0.7629	<0.0001	<0.0001	0.1819	0.1873	<0.0001	0.0107
	Population dynamics of *P. fuscipes*	0.0701	0.0013	0.0019	0.0542	0.2213	0.0008	0.3862
Wuxue	Percentages of folded leaves	<0.0001	0.0266	<0.0001	0.0002	<0.0001	0.0364	0.0003
	Population dynamics of *C. medinalis* larvae	<0.0001	0.0006	<0.0001	0.0027	<0.0001	0.0035	0.0125
	Population dynamics of *C. medinalis* adults	0.4678	0.0033	0.0053	0.2954	0.4166	0.0044	0.1755
	Population dynamics of *H. graminicola*	0.5090	<0.0001	<0.0001	0.9763	0.7571	<0.0001	0.9168
	Population dynamics of *U. insecticeps* per	0.6082	0.2390	0.0778	0.8626	0.6465	0.0300	0.8192
	Population dynamics of *P. pseudoannulata*	0.3614	0.3288	0.0007	0.7401	0.5980	0.4254	0.7043
	Population dynamics of *P. fuscipes*	0.7750	0.1586	0.8285	0.6194	0.4041	0.0001	0.8059

a) df=3, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. b) df=5, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. c) df=1, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. d) df=15, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. e) df=3, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. f) df=5, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year. g) df=15, 96 in 2009 and 2010 at Xiaogan, Suizhou and Wuxue for each year.

Table 2 Percentages of folded leaves (mean±SE) of the three transgenic *Bt* rice lines and non-transgenic Minghui 63 caused by *C. medinalis* at different days after transplanting at three sites in 2009 and 2010[a)]

Site	Year	Days after transplanting (d)	Percentages of folded leaves (%)			
			T1C-19	T2A-1	TT51	Minghui 63
Xiaogan	2009	38	0.00±0.00b	0.00±0.00b	0.00±0.00b	3.33±0.35a
		50	0.00±0.00b	0.00±0.00b	0.00±0.00b	1.00±0.61a
		63	0.00±0.00b	0.00±0.00b	0.00±0.00b	1.29±0.40a
		76	0.00±0.00b	0.01±0.01b	0.00±0.00b	1.15±0.42a
		87	0.06±0.06b	0.00±0.00b	0.00±0.00b	2.59±0.58a
		98	0.10±0.10ab	0.00±0.00b	0.00±0.00b	0.82±0.62a
	2010	34	0.00±0.00b	0.00±0.00b	0.00±0.00b	1.24±0.21a
		46	0.00±0.00b	0.00±0.00b	0.00±0.00b	3.66±0.27a
		58	0.36±0.18b	0.17±0.17b	0.00±0.00b	18.52±1.56a
		70	0.28±0.21b	0.28±0.21b	0.00±0.00b	22.09±1.12a
		82	0.16±0.08b	0.20±0.02b	0.00±0.00b	24.98±1.28a
		94	0.02±0.02b	0.04±0.04b	0.00±0.00b	23.57±1.99a
Suizhou	2009	43	0.00±0.00b	0.00±0.00b	0.07±0.07b	2.80±0.61a
		54	1.33±1.33b	0.80±0.42b	0.00±0.00b	11.93±2.04a
		65	5.20±0.99b	4.26±0.57b	0.22±0.22c	100.00±0.00a
		76	0.00±0.00b	0.05±0.05b	0.00±0.00b	100.00±0.00a
		87	0.00±0.00b	0.06±0.06b	0.00±0.00b	100.00±0.00a
		98	0.00±0.00b	0.07±0.07b	0.00±0.00b	100.00±0.00a
	2010	33	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
		44	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.07±0.00a
		56	0.07±0.07b	0.00±0.00b	0.00±0.00b	7.28±0.58a
		68	0.11±0.07b	0.57±0.53b	0.00±0.00b	9.70±1.65a
		83	0.06±0.03b	0.16±0.16b	0.00±0.00b	6.39±1.39a
		94	0.08±0.08b	0.14±0.14b	0.06±0.06b	6.33±1.37a
Wuxue	2009	32	3.07±0.98a	0.53±0.44a	12.00±12.00a	26.47±13.28a
		48	4.33±1.51b	3.33±0.48b	0.00±0.00c	30.60±7.90a
		60	2.53±0.58b	0.80±0.60b	0.33±0.24b	27.87±9.84a
		70	0.87±0.47b	0.40±0.20b	3.00±1.53b	61.07±5.04a
		82	0.93±0.33b	1.27±0.64b	3.53±2.34b	76.93±10.59a
		94	0.73±0.41b	0.73±0.37b	1.87±1.47b	70.40±10.98a
	2010	40	0.17±0.05b	0.06±0.03b	0.00±0.00b	4.11±1.56a
		50	0.84±0.12b	0.26±0.12bc	0.00±0.00c	12.90±1.03a
		60	0.00±0.00b	0.00±0.00b	0.00±0.00b	6.83±1.47a
		70	0.00±0.00a	0.00±0.00a	0.00±0.00a	2.08±2.08a
		82	0.17±0.13b	0.08±0.04b	0.23±0.09b	10.25±1.34a
		96	0.00±0.00b	0.00±0.00b	0.00±0.00b	5.97±1.27a

a) Means within a row indicated by different letters are significantly different (LSD test, d*f*=3, 8; $P<0.05$) among the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at the same site and the same year.

C. medinalis were also significantly higher on non-transgenic Minghui 63 compared with the three transgenic *Bt* rice lines at all sampling dates ($P<0.01$), except for those recorded at 33 DAT in 2010. Moreover, at Wuxue in 2009 and 2010, significantly higher percentages of folded leaves infested by *C. medinalis* were observed in non-transgenic Minghui 63 field compared with the three transgenic *Bt* rice field on all sampling dates ($P<0.01$), except at 32 DAT in 2009 ($P=0.3927$) and 70 DAT in 2010 ($P=0.4411$; Table 2).

2.2 Relative abundance of *C. medinalis* and its natural arthropod predators

Compared with non-transgenic Minghui 63, the transgenic rice had no significant impacts on the relative abundance of the four predators within the guild of predators at any site and at any sampling date throughout the studying time, except that a significantly higher relative abundance of *H. graminicola* was observed in the T2A-1 field compared with the non-transgenic Minghui 63 at Xiaogan in 2009 (Table 3). The relative abundance of *P. pseudoannulata* was also significantly higher in the T2A-1 field compared with Minghui 63 fields at Suizhou in 2009 (Table 3).

A significantly higher relative abundance of *C. medinalis* within the guild of phytophages was observed in the non-transgenic Minghui 63 field compared with the three transgenic *Bt* rice fields at any site and in any year (d*f*=3,8; $P<0.05$), with the exception of no significant difference in

Table 3 Relative abundance (mean±SE) of four species of arthropod predators on the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at three different sites in 2009 and 2010[a)]

Site	Year	Arthropod predator species	Rice line			
			T1C-19	T2A-1	TT51	Minghui 63
Xiaogan	2009	*H. graminicola*	23.56±2.52ab	33.22±4.20a	22.40±1.93ab	21.15±4.59b
		U. insecticeps	3.88±2.20a	3.40±0.54a	5.42±1.69a	5.40±0.93a
		P. pseudoannulata	15.72±2.17a	16.90±2.13a	11.40±1.72a	10.56±3.13a
		P. fuseipes	0.54±0.04a	0.59±0.40a	0.00±0.00a	0.55±0.10a
	2010	*H. graminicola*	14.34±3.35a	12.08±2.01a	10.01±1.88a	15.37±0.89a
		U. insecticeps	7.75±0.14a	6.03±1.46a	8.57±2.09a	8.17±2.69a
		P. pseudoannulata	3.29±1.62a	0.50±0.25a	2.19±0.73a	2.14±0.52a
		P. fuseipes	1.85±0.47a	0.64±0.36b	0.87±0.12ab	1.53±0.16ab
Suizhou	2009	*H. graminicola*	21.96±4.45a	26.89±2.48a	19.94±1.27a	21.23±3.05a
		U. insecticeps	1.54±1.31a	5.21±2.81a	2.75±1.18a	5.13±1.63a
		P. pseudoannulata	9.63±1.19ab	10.19±2.00a	5.17±0.98b	5.33±1.38b
		P. fuseipes	2.00±0.41a	0.73±0.44a	1.84±0.20a	2.06±0.79a
	2010	*H. graminicola*	27.81±3.06a	23.94±2.36a	27.59±1.62a	22.88±1.17a
		U. insecticeps	6.39±0.30a	4.99±0.69a	5.20±2.35a	5.32±0.66a
		P. pseudoannulata	1.49±0.74a	1.71±0.60a	1.35±0.22a	2.75±1.26a
		P. fuscipes	1.49±0.74a	0.36±0.36a	0.83±0.32a	0.61±0.38a
Wuxue	2009	*H. graminicola*	32.22±3.95a	31.14±9.00a	25.13±3.47a	21.54±5.68a
		U. insecticeps	0.61±0.42a	3.57±3.03a	1.72±1.01a	1.81±0.41a
		P. pseudoannulata	4.59±3.04a	2.17±0.45a	2.41±0.44a	1.89±0.38a
		P. fuscipes	0.75±0.34a	1.06±0.24a	0.66±0.33a	1.39±0.39a
	2010	*H. graminicola*	14.98±2.95a	7.63±2.49a	8.76±3.74a	11.95±1.38a
		U. insecticeps	1.26±0.22a	1.33±0.34a	0.35±0.35a	1.00±0.51a
		P. pseudoannulata	0.56±0.56a	0.00±0.00a	0.00±0.00a	0.00±0.00a
		P. fuscipes	1.95±1.40a	0.82±0.82a	2.42±0.87a	0.78±0.40a

a) Means within a row indicated with different letters are significantly different (LSD test, $P<0.05$) among the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at the same site and in the same year.

relative abundance recorded between Minghui 63 and TT-51 fields at Xiaogan in 2009 and at Suizhou in 2010 (Figure 1A).

2.3 Population densities of natural arthropod predators and *C. medinalis*

Compared with non-transgenic Minghui 63, the transgenic rice lines had no significant impacts on the population density of the four predators at any site and in any year (d*f*=3,8; $P>0.05$), with the exception of a significantly lower population density of *P. fuscipes* observed in the TT51 field at Xiaogan in 2009 and in the T2A-1 field at Xiaogan in 2010 compared with non-transgenic Minghui 63 fields (Table 4). The population density of *C. medinalis* (i.e., total number of *C. medinalis* collected with vacuum-suction machine and plant dissection) was significantly higher on the non-transgenic Minghui 63 compared with the three transgenic *Bt* rice lines at any site and in any year (d*f*=3,8; $P>0.05$), except that there was no significant difference in the population density of *C. medinalis* on Minghui 63 and TT-51 at Xiaogan in 2009 (Figure 1B).

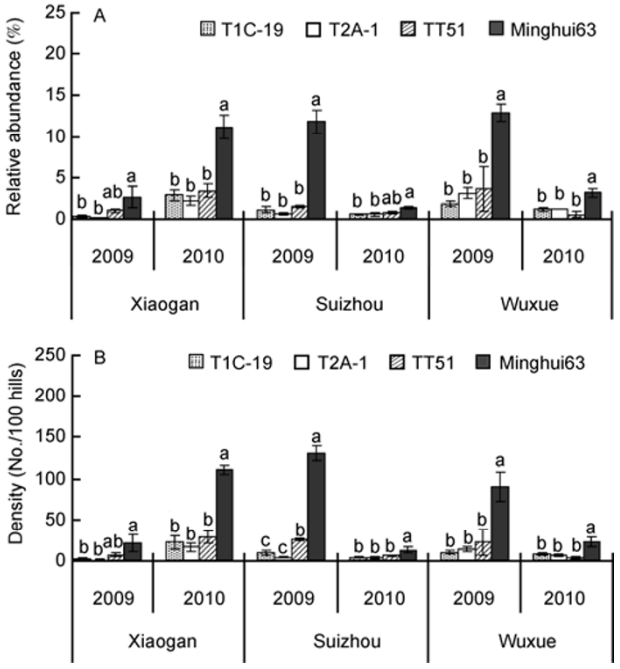

Figure 1 Relative abundance (A) and density (B) of *C. medinalis* on the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at different sites in 2009 and 2010. Bars labeled with different letters differed significantly (LSD test, $P<0.05$). Error bars indicate standard errors.

Table 4 Densities (mean±SE) of four species of arthropod predators (No./100 hills) on the three transgenic *Bt* rice and non-transgenic Minghui 63 at three different sites in 2009 and 2010[a)]

Site	Year	Arthropod predator species	Rice line			
			T1C-19	T2A-1	TT51	Minghui 63
Xiaogan	2009	*H. graminicola*	48.89±12.49a	64.72±19.00a	48.61±15.71a	48.89±11.49a
		U. insecticeps	7.78±3.86a	6.67±2.20a	10.28±1.94a	13.33±4.74a
		P. pseudoannulata	31.67±6.01a	30.00±2.20a	22.78±3.61a	26.94±12.23a
		P. fuscipes	1.11±0.28ab	0.83±0.48ab	0.00±0.00b	1.39±0.56a
	2010	*H. graminicola*	32.22±12.37a	30.83±0.00a	22.22±3.55a	33.33±2.89a
		U. insecticeps	16.39±2.65a	15.00±1.27a	19.17±4.74a	17.22±5.32a
		P. pseudoannulata	6.94±3.09a	1.11±0.56a	5.00±1.73a	4.72±1.39a
		P. fuscipes	3.61±0.56a	1.39±0.73b	1.94±0.28ab	3.33±0.48a
Suizhou	2009	*H. graminicola*	26.22±4.64b	28.67±1.02ab	45.56±8.75a	28.89±3.80ab
		U. insecticeps	1.78±1.46a	5.33±2.67a	5.33±1.33a	7.11±2.32a
		P. pseudoannulata	11.78±2.19a	10.89±1.82a	11.33±2.34a	7.11±1.46a
		P. fuscipes	2.44±0.59ab	0.89±0.59b	4.22±0.97a	2.89±1.24ab
	2010	*H. graminicola*	30.19±2.25a	25.74±2.87a	31.11±3.53a	27.59±2.73a
		U. insecticeps	7.04±0.67a	5.37±0.81a	6.11±2.85a	6.48±1.13a
		P. pseudoannulata	1.67±0.85a	1.85±0.67a	1.48±0.19a	3.15±1.34a
		P. fuscipes	1.67±0.85a	0.37±0.37a	0.93±0.37a	0.74±0.49a
Wuxue	2009	*H. graminicola*	46.67±7.98a	38.89±16.69a	44.26±11.87a	28.15±10.59a
		U. insecticeps	0.93±0.67a	4.07±3.28a	2.41±1.03a	2.04±0.19a
		P. pseudoannulata	6.48±4.26a	2.41±0.49a	3.89±0.32a	2.41±0.81a
		P. fuscipes	1.11±0.56a	1.30±0.49a	1.30±0.67a	1.67±0.32a
	2010	*H. graminicola*	13.06±3.20a	6.67±2.68a	6.94±2.90a	7.50±0.48a
		U. insecticeps	1.11±0.28a	1.11±0.28a	0.28±0.28a	0.56±0.56a
		P. pseudoannulata	0.56±0.56a	0.00±0.00a	0.00±0.00a	0.00±0.00a
		P. fuscipes	1.67±1.27a	0.83±0.83a	1.94±0.73a	0.56±0.28a

a) Means within a row indicated with different letters are significantly different (LSD test, $P<0.05$) among the three transgenic *Bt* rice and non-transgenic Minghui 63 at the same site and in the same year.

2.4 Population dynamics of *C. medinalis*

The population dynamics of *C. medinalis* larvae were significantly affected by rice line, sampling date, year, rice line×sampling date, rice line×year, sampling date×year, rice line×sampling date×year ($P<0.05$; Table 1). Sampling date, sampling date×year had significant effects on the population dynamics of *C. medinalis* adults at all the three experiment sites ($P<0.05$). However, there were no consistent significant effects of rice line, year, rice line×sampling date, rice line×year, rice line×sampling date×year on the population dynamics of *C. medinalis* adults at different experiment sites. For example, rice line and rice line×sampling date had significant influence on the population dynamics of *C. medinalis* adults at Xiaogan and Suizhou ($P<0.001$), but no significant impacts of rice line and rice line×sampling date were observed at Wuxue.

2.5 Population dynamics of natural arthropod predators

The population dynamics of the four natural predators were very similar among the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at Xiaogan (Figure 2), Suizhou (Figure 3) and Wuxue (Figure 4). The population dynamics of *H. graminicola*, *U. insecticeps*, *P. pseudoannulata* and *P. fuscipes* were not significantly affected by rice line, rice line×year, rice line×sampling date and rice line×sampling date×year at any sites ($P>0.05$), except that rice line× sampling date×year significantly influenced the population dynamics of *P. pseudoannulata* at Suizhou ($P<0.05$; Table 1).

However, there were no consistent significant effects of sampling date, year, sampling date×year on the population dynamics of *H. graminicola*, *U. insecticeps*, *P. pseudoannulata* and *P. fuscipes*. For example, sampling date had significant impacts on population dynamics of *P. fuscipes* at Suizhou ($P<0.05$), but no significant influence was observed at Xiaogan and Wuxue ($P<0.05$; Table 1).

3 Discussion

The rice leaffolder, *C. medinalis*, is an important rice pest in agroecosystems and is widely distributed across the main

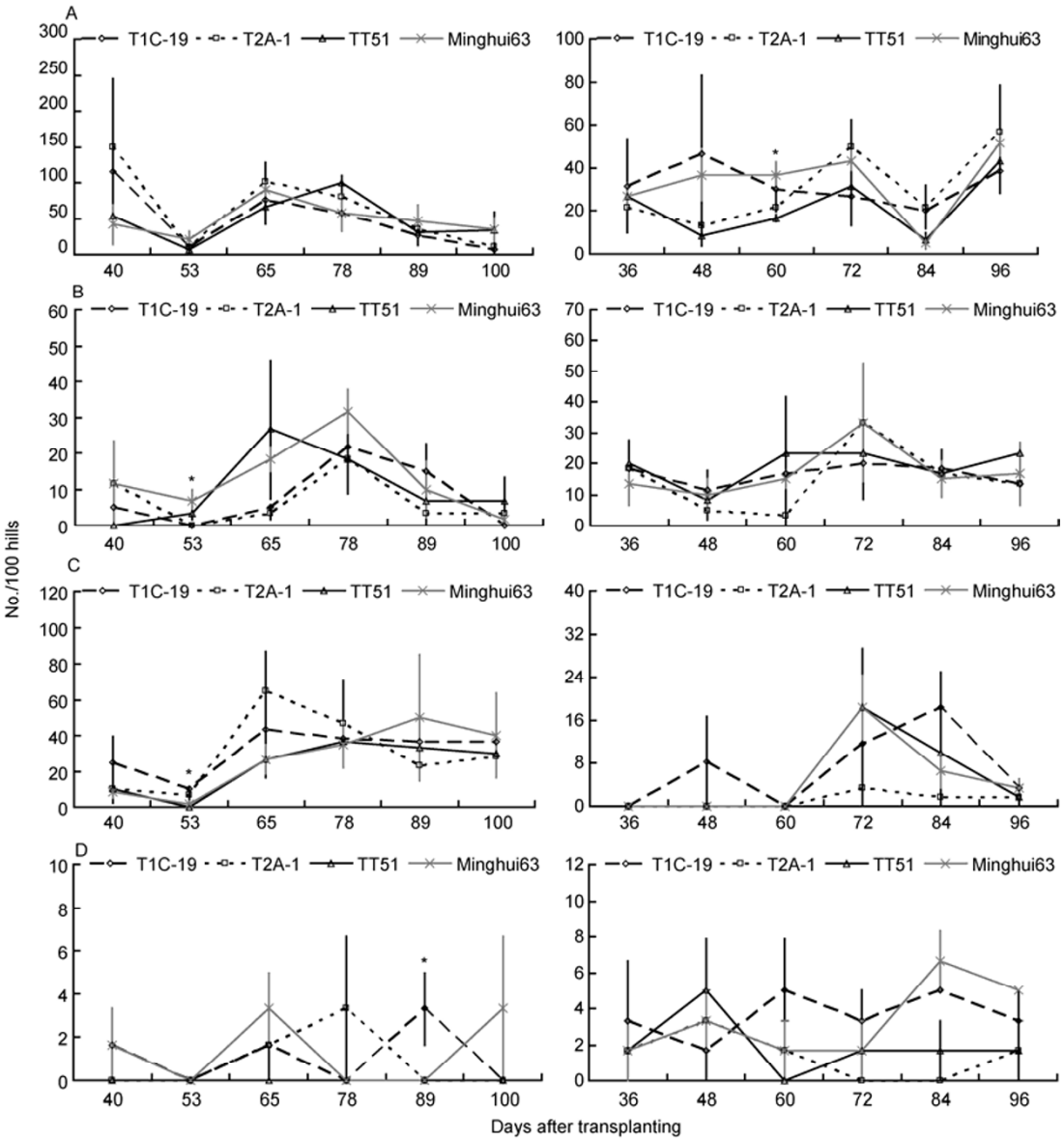

Figure 2 The population dynamics of the four arthropod predator arthropods (No./100 hills) on the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at Xiaogan site in 2009 (left) and 2010 (right). * indicates that there are significant differences among rice lines (P<0.05); otherwise, there are no significant differences among rice lines (LSD test, P<0.05). A, *H. graminicola.* B, *U. insecticeps*. C, *P. pseudoannulata.* D, *P. fuscipes*.

rice-growing areas of Asia [1]. It has frequent outbreaks and is responsible for, on average, a 5%−10% loss of rice yield. The loss can be as great as 60% during serious outbreaks [2, 24]. Transgenic *Bt* rice is a promising crop that can effectively control the infestation of these lepidopteran pests [25]. Many previous reports using laboratory or field tests have indicated that transgenic rice expressing *Bt* insecticidal protein genes are resistant to *C. medinalis* [26–30]. In our study, the percentages of folded leaves caused by *C. medinalis* significantly decreased on the three transgenic *Bt* rice lines compared with non-transgenic Minghui 63 rice except for four individual cases. From Table 2, the four exceptional cases could be caused by very low percentages of folded leaves (0%–0.82%) or high experimental variance (32 DAT at Wuxue in 2009). This suggested that the more severe the infestation caused by *C. medinalis*, the stronger the suppressive effects of transgenic *Bt* rice.

In the present study there was little, if any, effect of the transgenic *Bt* rice lines on the population density or population dynamics of the four natural arthropod predators compared with non-transgenic Minghui 63 rice. Likewise, the relative abundance of the four natural enemy arthropods within the predator guild was similar in the non-transgenic and transgenic *Bt* rice fields. Therefore, although the densities of *C. medinalis* were significantly lower in the transgenic *Bt* rice, the lower predator populations did not result from ap-

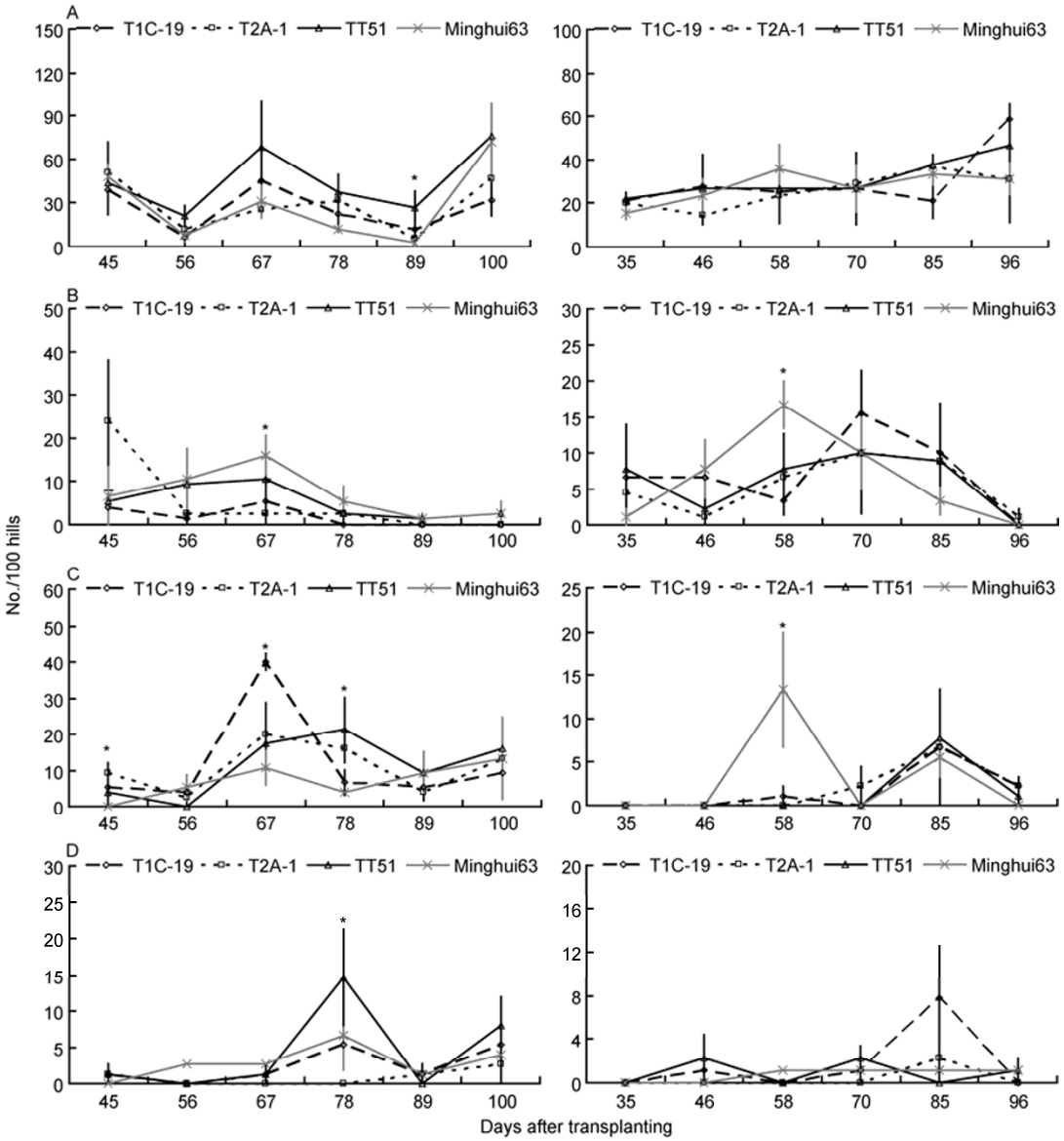

Figure 3 The population dynamics of the four natural predator arthropods (No./100 hills) on the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at Suizhou in 2009 (left) and 2010 (right). * indicates that there are significant differences among rice lines ($P<0.05$); otherwise, there are no significant differences among rice lines (LSD test, $P<0.05$). A, *H. graminicola.* B, *U. insecticeps*. C, *P. pseudoannulata.* D, *P. fuscipes*.

plication of *Bt* rice, a similar result to previous reports [10,18,31].

In most cases (75%), the population dynamics of *C. medinalis* adults were not affected by rice line, rice line×sampling date, rice line×year, rice line×sampling date×year, and no consistent effects of rice line, rice line×sampling date, rice line×year, rice line×sampling date×year on the population dynamics of *C. medinalis* adults were observed at different experiment sites. Whether this resulted from the sampling method, experimental variance or no antixenosis effect of *Bt* rice on *C. medinalis* adults or dispersal of moths needs to be further studied.

In China, agricultural biotechnology development strategies (e.g., Genetically Modified Organisms Breeding Major Projects) were carried out in 2008. In 2009, two transgenic *Bt* rice lines (TT51 and *Bt*-Shanyou 63) were awarded release certificates by Ministry of Agriculture of China, the first safety certificates for transgenic *Bt* rice in China. As an important staple food crop, an effective monitoring program of resistance evolution in transgenic *Bt* rice was performed to provide valuable and early warning information for the government and researchers [32]. Our studies have provided long-term and systemic evaluations of the effects of transgenic *Bt* rice on target lepidopteran pests and their natural arthropod enemy in the field, which has helped expedite the commercialization of transgenic *Bt* rice lines, such as TT51.

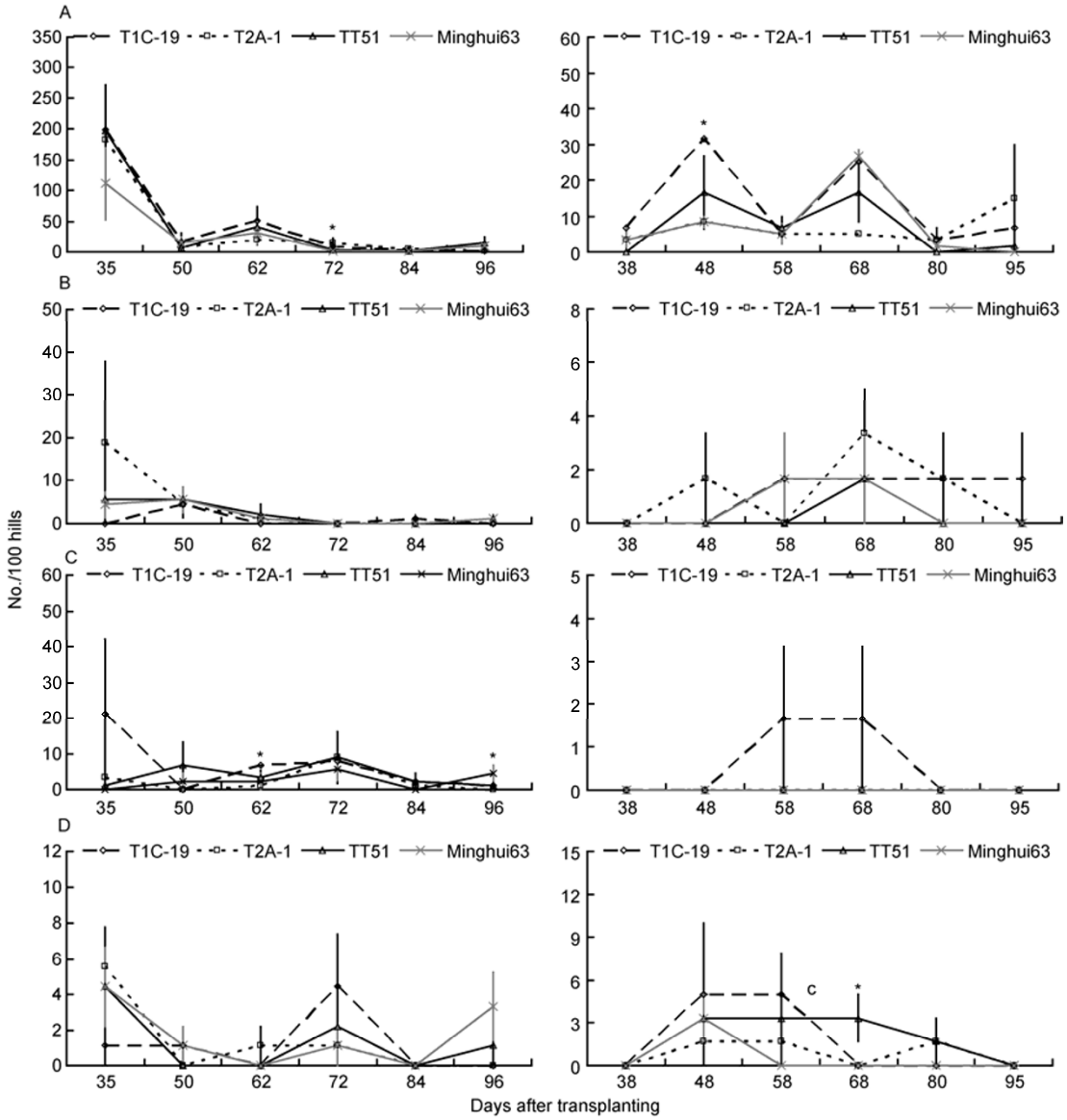

Figure 4 The population dynamics of the four natural predator arthropods (No./100 hills) on the three transgenic *Bt* rice lines and non-transgenic Minghui 63 at Wuxue in 2009 (left) and 2010 (right). * indicates that there are significant differences among rice lines (P<0.05); otherwise, there are no significant-differences among rice lines (LSD test, P<0.05). A, *H. graminicola.* B, *U. insecticeps*. C, *P. pseudoannulata.* D, *P. fuscipes*.

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